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Consequences of reduced vitamin A administration on mammary health of dairy ewes☆

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ABSTRACT

The study was carried out in Mytilene breed dairy ewes, which were allocated into two groups, 2 months before their first mating. All animals were given a diet based on dried alfalfa hay and concentrate compound feed with no added vitamin A or copper, hence making the diet poor in β-carotene and vitamin A. Ewes in group A were administered intramuscularly 150,000 IU of vitamin A at 3-month intervals, whilst animals in group B remained untreated. After lambing and on five occasions in total during their first lactation period, milk samples were collected from animals for somatic cell counting. Samples with somatic cell counts $\geq 0.5 \times 10^6$ cells mL^{-1}, as well as samples from clinical cases of mastitis were examined bacteriologically. Significantly ($P < 0.05$) fewer cases of clinical or subclinical mastitis were recorded in group A animals, compared to group B ones. Coagulase negative staphylococci were the most frequently isolated bacteria from secretion samples of ewes with clinical or subclinical mastitis. Somatic cell counts of milk of group A ewes were significantly smaller than those of group B on the first four sampling occasions ($P < 0.05$), but not on the one at the end of the lactation period ($P > 0.05$). It is suggested that vitamin A deficiency may lead to increased incidence risk of clinical and subclinical mastitis in and to increased milk somatic cell counts in dairy ewes.

1. Introduction

A protective effect of vitamin A on mammary health has been shown in studies carried out in mice (Chew et al., 1984) or dairy cows (Chew et al., 1982; Jukola et al., 1996; LeBlanc et al., 2004). In a previous study (Giadinis et al., 2011), we have reported that, in dairy ewes, increased incidence of clinical mastitis had been associated with reduced blood serum concentrations of vitamin A. However, there are no detailed studies available about the potential direct consequences of vitamin A deficiency in intramammary infection and mastitis in ewes. Objective of the present study was to investigate potential effects of vitamin A deficiency on the incidence risk of mastitis (clinical and subclinical) and the milk somatic cell counts of dairy ewes.

2. Materials and methods

Mytilene-breed ewe-lambs, on average 10-month-old, were included in the study. The animals were maintained indoors and given a daily diet

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Table 1
Chemical composition of the concentrate compound feed given to ewes during the study.

Parameter	Value
Dry matter	88.5%
Total proteins	17.5%
Digestible proteins	14.6%
Total fibres	4.8%
Calcium	1.2%
Phosphate	0.6%
Magnesium	0.3%
Sodium	0.4%
Energy	0.937 UFL
Vitamin A	0 μg kg^{-1}
β-Carotene	85 μg kg^{-1}

(per animal) as follows: 0.8 kg aged alfalfa hay, 0.3 kg wheat straw and 0.45–1.20 kg (depending on reproductive stage) concentrate compound feed (15% corn, 35% barley, 22% soya meal, 10% wheat, 13.5% wheat bran, 1.5% calcium carbonate, 0.5% salt and 2.5% of a vitamin–mineral premix with no vitamin A or copper). Chemical composition of the concentrate compound feed is in Table 1. Animals were allocated at random in two groups (A and B). Group A animals received 3500 IU vitamin A (as palmitate) kg^{-1} bodyweight (bw) every 3 months by intramuscular injection; group B animals were unsupplemented controls.

After 2 months into the study, all ewe-lambs received hormonal treatment, which included a combination of intravaginal administration of progestagens and intramuscular administration of equine chorionic gonadotrophin (Abecia et al., 2011, 2012). Rams were used for mating. Pregnancy was confirmed by ultrasonographic examination 3 months later (Fthenakis et al., 2012; Scott, 2012). Subsequently, ewes in the study lambed for the first time (group A $n = 25$, group B $n = 33$) and, following weaning of lambs, at the age of 6 weeks, they were machine-milked twice daily for another 15 weeks.

During that first lactation period, milk samples were collected from both mammary glands of all animals on the 3rd, 6th, 9th, 12th and 20th week of lactation, by using previously described sampling techniques (Fthenakis, 1994; Mavrogianni et al., 2005). All milk samples were submitted for somatic cell counting, by means of the Fossomatic® 5000 cell counting system (Foss Electric, Hillerod, Denmark). In samples with a cell count >0.5 × 10^6 cells mL^{-1} milk, as well as in secretion samples from cases of clinical mastitis a bacteriological examination was performed, by using established techniques (Barrow and Feltham, 1993; Euzeby, 1997).

Blood samples were also collected from all animals at lambing and on the 3rd, 6th, 12th and 20th week of their first lactation period. Vitamin A concentration in blood serum was measured by means of a colourimetric method (Roels and Trout, 1972), using a Hitachi 2000 Spectrophotometer (Hitachi Instruments Inc., Tokyo, Japan).

During the study, ewes with clinical mammary signs (e.g., abnormal secretion, enlargement, skin discolouration, and hardness) were considered to have clinical mastitis. Ewes without any clinically obvious abnormalities, but with milk, which contained over 0.5 × 10^6 cells mL^{-1} milk and, simultaneously, yielded over 400 c.f.u mL^{-1} at the microbiological examination, were considered to have subclinical mastitis. Analysis of results was performed using the statistical program SPSS v.17.0 for windows (SPSS Inc., Chicago, IL, USA).

3. Results

During the study, no animal developed clinical signs indicative of vitamin A deficiency. There were no significant differences in mean blood concentration of vitamin A between the two groups ($P > 0.05$) (Table 2).

In group A, 4 ewes (incidence risk: 17%) and, in group B, 11 ewes (incidence risk 39%) developed clinical mastitis ($P < 0.05$). The majority of the cases occurred during the 1st week *post-partum* (5/15) or the subsequent three weeks (8/15). Most bacterial isolates from cases of clinical mastitis were coagulase-negative staphylococci, isolated in pure or mixed culture; other isolates were coliform bacteria, *Mycoplasma* spp., *Staphylococcus aureus* and *Streptococcus* spp.

Table 2
Mean blood serum vitamin A concentration (μg mL^{-1}) of ewes supplemented (group A) or not (group B) with vitamin A.

Week of lactation	Group A	Group B
Lambing	0.32	0.32
3rd week	0.32	0.32
6th week	0.36	0.33
12th week	0.42	0.35
20th week	0.34	0.33

Differences between A and B were not significant ($P > 0.05$).

Results of somatic cell counts are in Table 3. Mean somatic cell counts were significantly increased in group B animals, in comparison to group A ewes, up to the 12th week of lactation ($P < 0.05$); no significant difference ($P > 0.05$) between the two groups was evident in the sampling performed on the 20th week of lactation. Moreover, a higher proportion of group B ewes was identified with subclinical mastitis during lactation (Table 4). Again, most bacterial isolates from cases of subclinical mastitis were coagulase-negative staphylococci (from 73% of affected mammary glands); other isolates were coliform bacteria, *Klebsiella* spp., *Mycoplasma* spp. and *S. aureus*.

4. Discussion

No significant difference in vitamin A blood concentration was evident between the two groups. The organism has an active homeostatic control in vitamin A (Thurnham and Northrop-Clewes, 1999), hence blood serum concentration is not a reliable indicator of vitamin A status, bar in cases of severe hypo- or hyper-vitaminosis A (Hidiroglou and Batra, 1995; Debier et al., 2005; Koutsoumpas et al., 2012). Moreover, the non-supplemented group did not develop clinical signs of vitamin A deficiency; Bolling et al. (1969) showed that turnover time for liver vitamin A was 234 ± 69 days, whilst Radostits et al. (2007) mentioned that adult sheep could live on a vitamin A deficient diet for 18 months before depletion of liver stores.

However, the findings indicate that vitamin A deprivation may have an impact on mammary health of dairy sheep. Vitamin A-deprived ewes had increased incidence risk and prevalence rate of clinical and subclinical mastitis cases, respectively. That is in accord with the findings of Giadinis et al. (2011), who have recorded, in flocks with increased (>10%) incidence risk of clinical mastitis, decreased blood serum vitamin A concentrations compared to flocks with smaller (<3%) clinical mastitis incidence. These authors have also reported that, within flocks with increased clinical mastitis incidence, sheep with mastitis had smaller vitamin A blood concentrations compared to healthy animals in the same flock. Apart from the above, no other publications have reported a potential association between vitamin A status and mammary health of ewes.

Somehow similar findings are available from work carried out in cows. Johnston and Chew (1984) found that cows